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Long-Term Accumulation of Hexachlorobenzene in Adipose Tissue of Parent and Filial Rats

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16. ABSTRACT

The concentrations of hexachlorobenzene (HCB) in adipose tissue were similar for F_0 and F_{1b} generations in rats fed 20 ppm HCB until 45 weeks of age. Nulliparous females receiving treatment equivalent to the HCB-treated F_0 generation rapidly accumulated HCB in their fat and, by 13 weeks of age, the residue values coalesced with values in 13-week-old F_{1a} females which had received additional HCB via the placenta and milk. Between 13 and 30 weeks of age, steady-state storage was approached and no significant increase in HCB concentrations occurred through 65 weeks of age. Postlactation dams, that nursed average size second litters had considerably lower concentrations of HCB in their fat than dams weaning no pups, suggesting substantial redistribution and/or elimination of maternal stores during lactation.

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LONG-TERM ACCUMULATION OF HEXACHLOROBENZENE IN ADIPOSE TISSUE OF PARENT AND FILIAL RATS

(Storage; lactation; maternal transfer)

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SUMMARY

The concentrations of hexachlorobenzene (HCB) in adipose tissue were similar for F rations in rats fed 20 ppm HCB until 45 weeks of age. Nulliparous females receiving treat to the HCB-treated F_0 generation rapidly accumulated HCB in their fat and, by 13 w residue values coalesced with values in 13-week-old F_{1a} females which had received add the placenta and milk. Between 13 and 30 weeks of age, steady-state storage was ap significant increase in HCB concentrations occurred through 65 weeks of age. Postlact nursed average size second litters, had considerably lower concentrations of HCB in their fat than dams weaning no pups, suggesting substantial redistribution and/or elimination of maternal stores during lactation.

INTRODUCTION

The widespread contamination of aquatic organisms, wildlife, meat, dairy products, and human fat and milk by HCB is well documented [1]. HCB toxicosis in humans has been substantiated in Turkish farmers who consumed flour made from seed grain containing HCB [2]. High mortality occurred in babies whose mothers had eaten the contaminated bread [3]. Consistent with the susceptibility of these children, Grant et al. [4] observed that suckling rats were especially vulnerable to HCB received in mother's milk. Additional evidence for the placental and lactational transfer of HCB has been reported by many investigators [1].

Abbreviation: HCB, hexachlorobenzene; pp'DDE, 1,1'-(dichloroethenylidene)bis[4-chlorobenzene].

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Transmittal of HCB through the placenta and the milk exemplifies exposure variants which may be encountered in the conventional multigeneration study. The parent generation (F_0) is usually of weanling age when dosing is begun, whereas filial generations are directly or indirectly exposed from the time of conception. As a result the daily dose rate, total dose, and manifestations of toxicity in parent and filial groups may differ in degree.

In a previous 3-generation HCB study in rats (unpublished data) we found that residue values for HCB in the fat of postlactation F_0 dams were 2–3 times higher than in dams of the subsequent F_1 and F_2 generations. This unexpected result could not be traced to error in feeding or analysis. Comparative data on males were not obtained so it was unclear as to what extent the discrepancy was influenced by gestation and lactation. The data also suggested the possibility that the perinatally exposed F_1 and F_2 animals were more efficient in eliminating HCB or metabolizing the compound. The present study was designed to verify the apparent differences in HCB storage between F_0 and F_1 generations. Although only one HCB dietary concentration and a control group were used, the experimental design replicated the original study in that the breeding and sacrifice schedules through termination of the F_{1b} adults were identical.

An addition to the modified replicate protocol consisted of feeding HCB to F_{1a} female offspring from both the HCB-treated and control groups. Some of the F_{1a} control females were provided the HCB diet starting at 5 weeks of age so that the HCB exposure regimen in these animals was equivalent to the HCB-treated F_0 generation. This allowed residues to be compared agewise in perinatally and nonperinatally exposed females with otherwise identical environmental backgrounds, and avoided the complicating factors associated with parity.

METHODS

The experimental schedule is illustrated by the diagram in Fig. 1. Parent groups (F_0), each consisting of 10 male and 15 female Sherman strain 5-week-old rats were fed either powdered plain chow (controls) or a powdered diet containing 20 ppm technical grade (94%) HCB. The rats were bred when approx. 14 and 30 weeks of age to produce the F_{1a} and F_{1b} litters and killed approx. 45 days after weaning of the second litters. 10 males and females from the F_{1b} litters were continued on the parental diets from weaning and bred and killed at ages corresponding to the F_0. Perirenal fat was collected for residue analysis from rats fed HCB.

For comparisons of HCB concentrations vs. age in perinatally and non-perinatally exposed females it was desirable to use animals with otherwise identical backgrounds; therefore, the F_{1a} offspring of both control and treated groups were utilized in the following manner. The F_{1a} litters were culled to 10 pups at birth and at 21 days (weaning) female pups from both the control and 20 ppm HCB-treated groups were selected randomly from all available litters. Subgroups of F_{1a} females

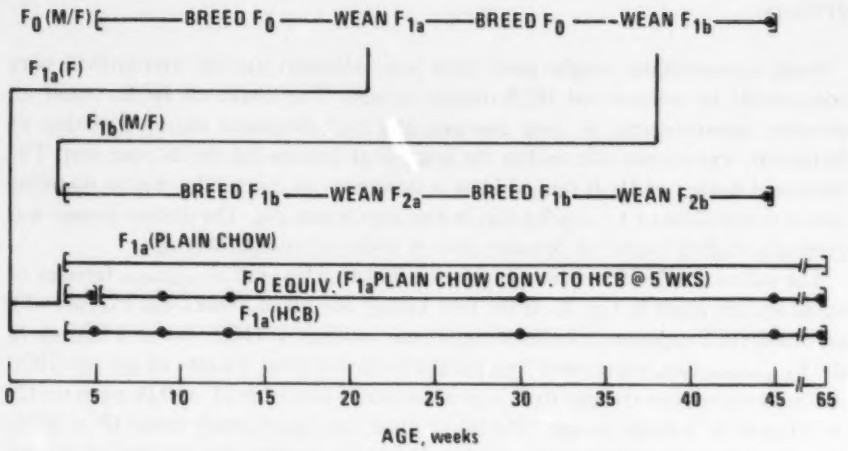

Fig. 1. Approximate ages of rats relative to treatment, breeding, and residue measurement schedules. F_0 and F_{1b} males and females, and F_{1a} females were fed powdered plain chow or 20 ppm HCB continuously within the intervals indicated by brackets. ●, killing for residue measurements (HCB-treated only).

whose parents had been fed plain chow or HCB were continued on the parental diets from weaning. A third group of F_{1a} females whose parents had been fed plain chow was converted to the 20 ppm HCB diet starting at 5 weeks of age so that the exposure schedule in this group ($F_{0\ equivalent}$) was equivalent to the HCB-treated F_0 generation. 4 rats from the subgroups fed HCB were killed when the rats were 5 (pretreatment for $F_{0\ equivalent}$), 9, 13, 30, 45 and 65 weeks of age. Perirenal fat was collected for residue analysis.

All rats were weighed biweekly. Food consumption was measured periodically, including the first week of dietary dosing, the week before each breeding, and the week before each killing. At necropsy, the livers of females fed HCB were examined under long-wave UV light for gross evidence of excess porphyrins [5]. HCB residues were not measured in animals fed only plain chow. These animals were kept for monitoring food consumption, weight gain and reproductive parameters (litter size, viability and growth).

The HCB concentrations in adipose tissue were determined as previously described [6]. HCB concentrations in the F_{1a} and $F_{0\ equivalent}$ females were fit to a two-factor linear model and compared by contrasts of the fitted function. The factors were exposure regimen (perinatal plus postweaning or postweaning only) and age at the time of killing. To protect against multiple testing errors, the Bonferroni Bound procedure was used for determining significance levels.

RESULTS

Food consumption, weight gain, litter size, neonatal survival and growth were comparable in control and HCB-treated groups. The intake of HCB, based on periodic measurements of food consumption (not measured during gestation or lactation), was comparable within the sexes in all groups fed the 20 ppm diet. The measured dosage of HCB ranged from a maximum of 2.4 mg/kg/day in weanling rats to a minimum of 1.1 mg/kg/day in rats over a year old. The dietary dosage was generally slightly higher in females than in males of comparable age.

The values for HCB residues in adipose tissue of F_{1a} and $F_{0\ equivalent}$ females of equal age are given in Fig. 2. At the first killing, the fat of 5-week-old F_{1a} rats with perinatal HCB exposure contained significant residues of HCB (441 ± 15 ppm). In the $F_{0\ equivalent}$ rats, which were first fed the HCB diet when 5 weeks of age, the HCB concentrations rose sharply from a pretreatment value of 0.53 ± 0.06 ppm to 329 ± 18 ppm at 9 weeks of age. The latter value was significantly lower ($P < 0.05$) than the 394 ± 16 ppm found in the 9-week-old F_{1a} females that received additional HCB perinatally. The residue values in the two groups coalesced by 13 weeks of age, then increased in parallel between 13 and 30 weeks of age ($P < 0.05$). No significant increase in HCB concentrations occurred between 30 and 65 weeks of age.

Compared with the residue values in the nonparous females of similar age, the HCB concentrations in the fat of 45-week-old males were roughly 30% lower, the values being 409 ± 20 and 415 ± 11 ppm (N = 4) for F_0 and F_{1b} males, respectively.

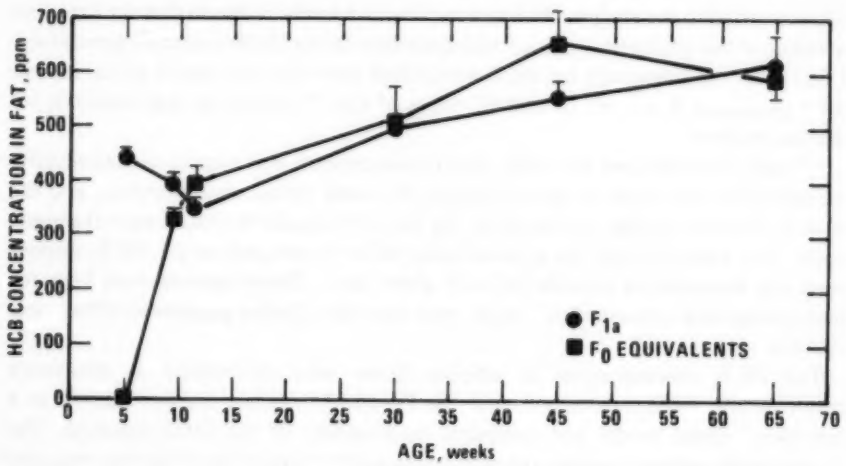

Fig. 2. HCB concentrations in adipose tissues of $F_{0\ equivalent}$ and F_{1a} female rats exposed to 20 ppm HCB in the diet. Points are means ± SEM for 4 rats.

TABLE I

HCB RESIDUES IN ADIPOSE TISSUE OF POSTLACTATION F_0 AND F_{1b} DAMS FED 20 ppm HCB[a]

Maternal generation	Pups weaned		HCB concentration in maternal fat ppm
	First litter N	Second litter N	
F_0	8	0 (NP)[b]	554
	10	0 (NP)	491
	10	0 (NP)	469
	9	9	268
	9	8	260
	10	9	249
	6	8	245
	10	11	236
F_{1b}	0 (NP)	0 (NP)	513
	10	0 (NP)	488
	12	0	484
	8	0	471
	0	3	469
	11	0	430
	5	1	357
	0 (NP)	5	320
	14	3	293
	0 (NP)	9	271

[a]Figures are values for individual dams. Both F_0 and F_{1b} dams were killed when 45 weeks of age, approx. 45 days after weaning their second litters.
[b]NP, dams not pregnant.

The HCB concentrations in the fat of F_0 and F_{1b} dams approx. 45 days after weaning their second litter are given in Table I. The data did not permit correlation of maternal residues with litter size; however, in dams that weaned no pups, or were not pregnant in the second breeding, the concentrations of HCB were nearly 2-fold higher (486 ± 12 ppm) than in dams that weaned one or more pups (297 ± 23 ppm).

Black areas of discoloration on the liver were observed in 4 HCB-treated females (3 $F_{0\ equivalent}$ and one F_{1a}) killed at 45 or 65 weeks of age. Under UV light, the dark areas exhibited red fluorescence characteristic of excess accumulation of porphyrins [5].

DISCUSSION

The HCB concentrations in adipose tissue were comparable in adult F_0 and F_1 animals that received equivalent HCB dosage after 5 weeks of age. Therefore, the

steady-state concentrations of HCB were unaltered by the additional prenatal and neonatal exposure received by the F_1 animals.

Mendoza et al. [7] have reported that significant residues of HCB in rat pups can be acquired from the mother's milk. Supporting data have been presented by Grant et al. [4] who found HCB concentrations of 527 ppm in the fat of 21-day-old female rats whose parents had been fed 20 ppm HCB. In the present study, the first residue measurement in the F_{1a} females with perinatal HCB exposure was made at 5 weeks of age. At this point in time, these animals had eaten the 20 ppm HCB diet exclusively for 2 weeks and may also have eaten some of the diet prior to weaning. However, the mean HCB concentration of 441 ppm at 5 weeks was higher than expected, had the 20 ppm diet been the only source of HCB. It is probable that a significant portion of the residue measured at 5 weeks was obtained from the milk. Examination of Fig. 2 suggests a trend toward declining HCB concentrations between the ages of 5 and 13 weeks in perinatally exposed F_{1a} animals. HCB stored prior to 5 weeks may have been partly eliminated, or diluted by the rapidly increasing lipid pool of the growing animals.

HCB residues in the perirenal fat of dams which had nursed average size second litters were appreciably less than in dams which had lost their litter or had failed to become pregnant in the second breeding. A few data points available from other studies at this laboratory (unpublished data) are consistent with this finding. Analogous results have been reported by Takagi et al. [8] who studied the maternal transfer of a polychlorinated biphenyl mixture in rats. These authors found that, with intermittent intake (weekly oral doses), the concentrations of polychlorinated biphenyls in organs of nursing dams were markedly lower than in nonpregnant animals. Since food intake in the rat increases by about 250% during lactation [9], the lactating dams in the present study substantially increased their daily intake of HCB. The decreased HCB concentrations in the postlactation dams show that increased intake did not prevent appreciable redistribution and/or elimination of maternal stores.

It has been common practice in multigeneration studies in rodents to discard the first litters of each generation at weaning and rebreed immediately or after a short resting period. Offspring from the second litters are usually selected for further breeding. In the present study, the postlactation interval of 45 days was insufficient time for HCB concentrations in the dams to rebound to levels expected, had the dams not nursed full litters. The lower HCB concentrations suggest that the contribution to the milk from maternal stores can be substantial, even though maternal storage approaches steady state and maternal intake is increased during lactation. The results also suggest the potential for nonequivalent exposure in successive litters within a generation. If depletion of maternal stores results in less HCB being excreted in the milk, insufficient resting time between breedings could result in second and subsequent litters receiving a smaller cumulative dose than first litters.

Adamović et al. [10] concluded that the intake of pp′DDE by breast-fed infants can exceed amounts attributable to the nursing mother's daily dietary intake. Vodicnik and Lech [11] have shown conclusively that essentially 100% of an absorbed single maternal dose of 2,4,5-2′,4′,5′-hexachlorobiphenyl can be transferred via the milk to suckling mice. Further studies are necessary to determine to what extent maternal stores of HCB and other compounds can be purged via the milk when maternal intake is constant or increased during lactation. In a broader sense, the transfer of toxic chemicals from mother to offspring is a complex aspect of reproductive toxicology with important implications for both testing and populations at risk.

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